Indian Journal of Ecology (2021) 48 Special Issue (13): 43-46

Manuscript Number: S-527
NAAS Rating: 4.96

Purifying, Characterizing and Evaluation of Antioxidant Efficacy of Goat Milk Lactoferrin

Asaad Shamil Atiya, Shabeeb Munshid Jasim and Abdulridha Ati Jaafar

College of Agriculture, University of Misan, Misan, Iraq
E-mail: asaadshameel1986@gmail.com

Abstract: The present study included isolation, extraction, and purification of lactoferrin protein from goat milk whey using ÄKTA Pure 25 and separation column Superdex 200 10/300 GL. A single peak appeared on 280 nm and at a flow rate of 0.5 ml/min. This peak was collected inside test tubes and kept at a 4°. The samples were submitted to electrophoresis technique by using polyacrylamide gel and the presence of dirtied materials (SDS-PAGM), one band was obtained with molecular weight reached to 75.20 Kd, while the concentration of purified Lactoferrin was 0.67 mg/ml with 6.74% carbohydrate 9.4% and 168 ppm iron. The highest antioxidant efficacy of purified lactoferrin was 81.76% at 25 mg/ml concentration, whereas α-Tocopherol reported 67.24% antioxidant efficacy at the same concentration. All concentrations of purified lactoferrin showed significant anti-oxidant efficacy in comparison with other concentrations of the natural antioxidant α-Tocopherol .BHT (industrial antioxidant) in all concentrations was superior to both lactoferrin and α -Tocopherol and recorded 95.23% at the same concentration (25 mg/ml). The purified lactoferrin portent of the goat milk whey was significant as an anti-oxidant in comparison with α-Tocopherol at all the concentrations.

Keywords: Antioxidants, Lactoferrin Goat milk, Lactoferrin

Goat's milk proteins have gained increasing attention, especially of the proteins and peptides that hydrolysis and bioactive, including lactoferrin. The interest in bioactive of goat's milk is intensifying due to its reduced allergenicity compared to bovine milk and is approaching from human milk , because it does not contain on ß-lactoglobulin protein which is considered the first responsible of allergenicity of milk in children (Ahmed et al 2015). In addition to goat milk contain on an important vitamins and minerals like vitamin C, therefore it is a good source of food. The cow's milk its acidity develops and curd in 48 hours, while goat's milk needs to longer time to curd, because goat milk contains on anticoagulant compounds, including lactoferrin. The use of natural resources is safer to maintain the public health, which has made researchers and specialists to move towards their use and avoid chemicals in treating diseases, due to secondary complications and side effects of these substances. Lactoferrin protein is one of the materials naturally present in many secretions and natural fluids, which plays a fundamental role in supporting the immune system through its ability to bind iron and prevent the formation of free radicals (Al-Hatim 2012)

Lactoferrin is one of the basic milk whey glycoprotein and play a major role in the inhibition of fats and oils peroxides. The hydrolysis peptides of lactoferrin such as lactoferrin, lactoforein and lactofermpin , has a major role to combat many diseases such cancers, inflammation, and allergies, due to their high vital effectiveness in hindering the growth of microorganisms and inhibiting peroxides (Kanwar et al 2015). The quantities of whey that are released as waste during the dairy industry are dangerous pollutants for public health and environmental pollution and growth of microorganisms on them. In addition to these has a high nutritional value because they contain essential amino acids for human body and therefore are considered of economic importance. In world about 145 million tons annually from the whey and 30261.6 tons from Iraq which is dumped as a waste during the manufacture of cheese. Therefore, developed countries have introduced them to the food industries, such as fortifying baby food, making pastries, pharmaceutical industry, cosmetics, and making biofilms that encapsulate and support some food products, such as biofilms made from whey proteins.(Aziz 2001)..The current study aimed to isolate and purified lactoferrin from goat milk whey and study the antioxidant efficacy of the isolated lactoferrin.

MATERIAL AND METHODS

The fat and caseins were isolated from goat milk to gain the whey, according to the method of Levieux et al (2006) and then dialysis was implemented by dialysis bags with molecular weight between 5-10 Kd. Freeze drying was conducted to concentrate whey samples and kept at 4° until use. Gel filtration for purification of lactoferrin protein from

goat milk whey was conducted by using ÄKTA Pure 25 and separation Colum Superdex 200 10/300 GL at flow rate 0.5 mL/min., under 1.5 MPa pressure and 280 nm wavelength. Each peak was collected by the F9-R at 2 mL/part. All samples were submitted to electrophoresis technique by using polyacrylamide gel and the presence of dirtied materials (SDS-PAGEM) for detection of protein purification and molecular weight for lactoferrin.(AL-Taai 2017). The carbohydrate content was estimated according to the method of Dubois et al (1956) and protein content was estimated as described by Lowry et al (1951). Lee and Clydesdale method (1979) was used to estimate the total iron of the purified lactofrein protein.

Estimation of antioxidant efficacy: According to the procedure provided by Osawa and Namiki (1981) the anti-oxidant effect of the ferric thiocyanate was measured with some modifications and the method included mixing of an 8 mL mixture of the buffer phosphate 0.05M and pH 7 with 4.1mL of linoleic acid at 2.5% concentration ethanol with the addition of 4 mL of lactoferrin protein and 3.9 ml distilled water. Then the mixture was incubated at 40 ° for 24 hr. then and 0.1 ml of the incubated mixture was added to 9.7 ml ethanol with a concentration of 75% and 0.1 ammonium thiocyanate with a concentration of 30% and 0.1 of ferrous chloride of 0.02 M (0.07 g in 20 ml of hydrochloric acid with a concentration of 3.5%) and then shake gently. The antioxidant efficacy was measured at a wavelength of 500nm.

$$Inhibition = \{1 - \frac{(Abs\ control)}{Abs\,\text{model}}\} \times 100 \quad Abs : Absorption$$

Samples of the control group were prepared in the same way above, by replacing protein sample with 4 mL ethanol. The purified samples of lactoferrin have been were compared with BHT, α -Tocopherol and control group at a concentration ranging from 5-25 mg/ml.

RESULTS AND DISCUSSION

The fatty layer of goat milk was isolated at a speed of 4500 - 5,000 rpm for 30 minutes using a refrigerated centrifuge. The speed was then increased to 10,000 - 20,000 rpm for 20 minutes after the addition of diluted HCL droplets until reaching to electric point 4.3 to separate small, medium and large casein of goat milk and the goat milk whey was obtained. Then partial purification was carried out by using a dialysis bags. The result agree with AL-Hatim (2012).

Ionic exchange: The results of ion exchange chromatography which has been representing the most common method used for purifying milk proteins, showed the ion exchange of goat milk whey along the 280 nm wavelength at a 3-3.5 ml/min flow rate. The first peak representing the lactoperoxidase protein at 0.2 M salt gradients of NaCl. The second peak with the pink-color represented lactoferrin protein, the pink color attributed to the conjugation of lactoferrin to iron ions and the highest absorption point was 0.49 along the wavelength of 465 nm (Fig. 1). These findings are consistent with results of Abbas (2015).

Gel filtration to purify lactoferrin: The purification of the lactoferrin protein from the goat milk whey after the ion exchange phase, the second isolated peak was taken from the ion exchange phase and injected in the ÄKTA pure 25 on the supersdex 200 10/300 GL. Isolation conditions were fixed on the 280nm wavelength at 0.5 mL/min and 1.5-2 MPa pressure using the basic tris-HCL with pH 8.7 and 0.02 m concentration to get the whole purification of the isolated parts of the goat's milk whey, one peak with a maximum absorption of 191 mAU was appeared at 14.34 minutes (Fig. 2) and these results are in accordance with Abbas (2015) and AL-Rikabi et al (2016).

Electrophoresis with presence of dirtied materials (SDS-PAGM): The results of electrophoresis by using polyacrylamide gel and the presence of materials (SDS-PAGM) for the purified lactoferrin protein in the ion exchange and gel filtration to determine the molecular weight of lactoferrin goat milk whey in comparison with standard proteins after calculation of the proportional movement. One band was obtained with molecular weight of 75.20 Kd (Fig. 3) which indicates that the protein has reached a high purity level and these results are agree with Abbs(2015) and Alluwaimi et al (2017).

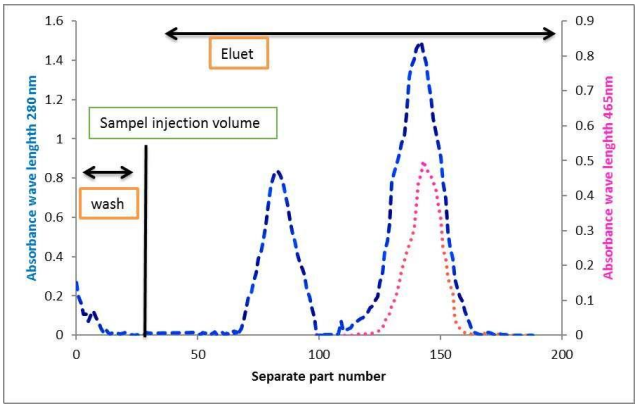

Fig. 1. Chromatography ionic exchange of goat milk whey using cation ionic exchange cm-sephadex C-25, dimension 1.5 x 60 cm, At flow rate of 3.5 ml/min, the column was balanced with the Tris-HCL solution of 0.05 m and pH 7.8. The first phase of the recovery (ELUTE) was represented by using of the tris-HCL of 0.05 m, PH 7.8 which was contain NaCl of 0.2 m and 0.5 m of NaCl was used in the second phase of recovery

Lactoferrin concentration: Dialysis bags were used for 24 hr. in two phases which have molecular weights of 5,000 Kd and 7 Kd for partial purification of whey proteins, and buffer solution of the Tris-HCL with pH 8.6 and 0.02 M for maintaining the pH of Lactoferrin. Then all samples were submitted to freeze drying until reaching to 0.68 mg/ml after the ion exchange phase in lactoferrin of goat milk whey and 0.83 mg/ml at the end of the purification phase, as its shown in Table 2 (Hiss et al 2008).

Content of lactoferrin carbohydrates: The content of lactoferrin carbohydrates was 6.74% and these results were consistent with the Shimazaki (2000) and observed that carbohydrate of cows lactoferrin ranged between 7-11.5% .

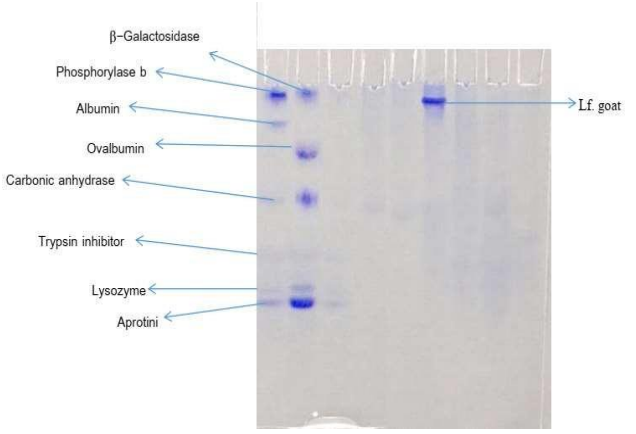

Fig. 2. Gel filtration for the second peak of the ionic exchange of goat milk whey using the Äkta Pure 25, on the Superdex 200 10/300 GL at flow rate of 0.5 ml/min on 280 nm

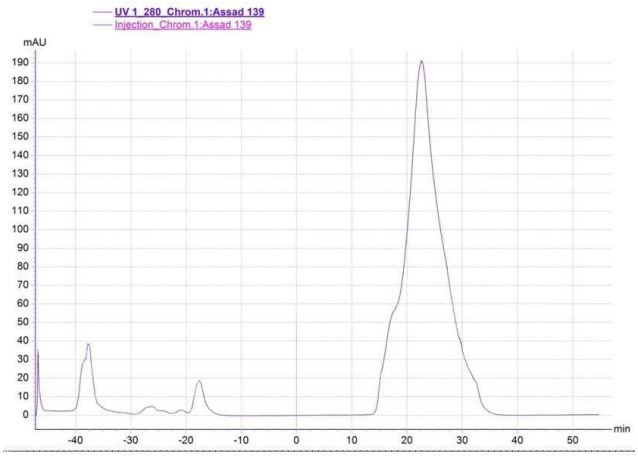

Fig. 3. Shows the Electrophoresis by using polyacrylamide gel and the presence of dirtied materials(SDS-PAGM) and2-mercptoethanol to estimate molecular weight and confirm the purity of lactoferrin goat milk whey at 12% concentration

Total iron content of lactoferrin protein: The level of saturated iron in purified lactoferrin protein of goat milk whey was 9.4% and 168 ppm (Table 3) and this result is due to the high content of carbohydrate for lactoferrin protein of goat milk. This indicated a greater potential of lactoferrin protein for binding free iron in the center especially with the greater openness in C lobs of protein (Baker & Baker 2005). These results are in line with Shimazaki (2000), who found observed that the rate of saturated iron in the camels lactoferrin was 9 %. Aziz said (2001) reported that the contents of the saturated iron in cows and buffalos lactoferrin are 13.85 and 18.98%, respectively.

Antioxidant efficiency: High antioxidant effectiveness of lactoferrin of goat milk whey for linoleic acid peroxides was 81.76% at 25 mg/mL (Fig. 4). Earlier workers also observed similar trend (Simos et al 2011, Habib et al 2013, Ahmed et al 2015). This variation are in the anti-oxidant effect to protein lactoferrin are due to different sources, materials, and purification devices, as well as a variety in the quantity and quality of protein in addition to the differences of molecular weight and the number and locations of amino acids involved in protein structure .

The highest anti-oxidant effect for linoleic acid peroxides 95.23% at 25 mg/ml was in BHT group, while the highest anti-oxidant effect of α -Tocopherol was 74.27% at the same concentration. These findings are close to those of the Al-Hatim (2012). The statistical analysis showed significant increase in the antioxidant of the industrial BHT in all concentrations in comparison to lactoferrin and α -Tocopherol. The lactoferrin exhibited high significant antioxidant effect as compared to α -Tocopherol. The highest antioxidant effect of lactoferrin of goat milk whey is due to the high capacity to give hydrogen electrons and binding iron ions with lactofeerin leading to chelate the free radicals in addition to the differences of the number and locations of

Table 1. Standard proteins used in electrophoresis technique by polyacrylamide gel and the presence of dirtied materials (SDS-PAGM)

Source	Molecular weight (kilo dalton)	Standard protein name
A. *coli*	116	β-Galactosidase
Rabbit muscle	97.4	Phosphorylase b
Bovine serum	66	Albumin
Chicken egg white	45	Ovalbumin
Bovine erythrocytes	29	Carbonic anhydrase
Soybean	20.1	Trypsin inhibitor
Chicken egg white	14.4	Lysozyme
Bovine lung	6.5	Aprotinin

Table 2. Concentration of protein lactoferrin purfed of goat milk whey and content iron

Stages of the treatment of the lactoferrin	Goat
After the ion exchange	0.68 Mg/ml
After gel filtration	0.83 Mg/ml
Total iron content	Part in % 9.4, 168 million
Carbohydrates	6.74%

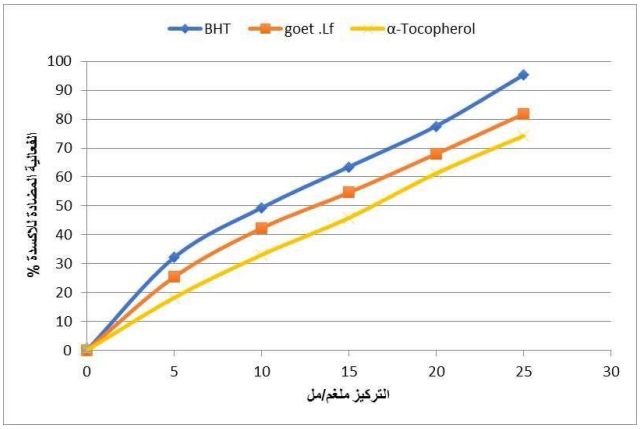

Fig 4. Antioxidant efficacy of a lactofreen protein debuster compared to the BHT and α-tropherol

amino acids involved in proteins molecule structure as well as the differences in the number and locations of hydroxyl groups in the amino acids structure (Pihlanto 2006). The anti-oxidant effect of lactoferrin of goat milk whey compared with the normal and industrial antioxidants might be attributed to the difference in the material, purification methods and type of solvent used for isolation, thus lactoferrin possess properties depends on molecular structure and presence of hydroxyl and ketone groups in half of each rings with the reciprocal double-bonds.

REFERENCES

Abbas ZH 2015. *Isolation, Purification and Characterization of Lactoferrin From Goats Colostrum and Study Its Anticancer Activity Against Some Cancer Cell Lines.* Dissertation Submitted, University of Baghdad-Iraq **189**.

Ahmed AS, El-Bassiony T, Elmalt LM and Ibrahim HR 2015. Identification of potent antioxidant bioactive peptides from goat milk proteins. *Food Research International* **74**: 80-88.

Al-Hatim Raqad Rahim Ali 2012. *Isolation and pure the lactoferrin protein from colostrum of bovine and ovine and study some of its properties and applications.* Master Thesis, College of Agriculture, Basra University, Iraq, pp. 143.

Alluwaimi AM, Salem KTA, Al-Ashqer RA and Al-shubaith IH 2017. The Camel's (*Camelus dromedarius*) Mammary Gland Immune System in Health and Disease. *Journal of Advance Dairy Research* **5**: 171.

Al-Rukabi Ali Khudair, Aziz Raghad Akram and Al-Hatem Raghad Rahim Ali 2016. Study the anti-microbial and oxidative activity of lactoferrin protein purified from cow and sheep colostrum. *Dhi Qar University Journal of Agricultural Research* **4**(2): 216-230.

AL-Taai Iman Hadi Auda 2017. *Extraction and Purification and Characterization of Alliinase from Iraqi Garlic (Allium sativum) and Using its Products to Preparation A Food Supplement.* Master Thesis, College of Agriculture, Basra University, Iraq, pp. 123.

Aziz Ragad Akram 2001. *Study the activity of lactoferrin protein separated colostrum from bovine and buffalo.* Master Thesis, College of Agriculture, University of Baghdad, Iraq, pp. 126.

Baker EN and Baker HM 2005. Molecular structure, binding properties and dynamics of lactoferrin. *Cellular and Molecular Life Sciences* **62**(22): 2531-2539.

Habib HM, Ibrahim WH, Schneider-Stock R and Hassan HM 2013. Camel milk lactoferrin reduces the proliferation of colorectal cancer cells and exerts antioxidant and DNA damage inhibitory activities. *Food Chemistry* **141**: 148-152

Hiss S, Meyer T and Sauerwein H 2008. Lactoferrin concentrations in goat milk throughout lactation. *Small Ruminant Research* **80**: 87-90.

Kanwar Jagat R, Kislay Roy, Yogesh Patel, Shu-Feng Zhou, Manju Rawat Singh, Deependra Singh, Muhammad Nasir, Rakesh Sehgal, Alka Sehgal, Ram Sarup Singh and Sanjay Garg 2015. Multifunctional iron bound lactoferrin and nanomedicinal approaches to enhance its bioactive functions. Academic editor: *Didier Astruc Molecules* **20**: 9703-9731

Levieux D, Levieux A, El-Hatmi H and Rigaudie`re J 2006. Immunochemical quantification of heat denaturation of camel (*Camelus dromedaries*) whey proteins. *Journal Dairy Research* **73**: 1-9.

Osawa T and Namiki M 1981. A novel type of antioxidant isolated from leaf wax of Eucalyptus leaves. *Agricultural and Biological Chemistry* **45**: 735-739.

Pihlanto A 2006. Antioxidative peptides derived from milk proteins. *International Dairy Journal* **16**: 1306-1314.

Shimazaki K 2000. Lactoferrin: A marvelous protein in milk?. *Animal Science Journal* **71**: 329-347.

Van-Veen HA, Geerts MEJ, Van Berkel PHC and Nuijens JH 2004. The role of N-linked glycosylation in the protection of human and bovine lactoferrin against tryptic proteolysis. *European Journal of Biochemistry* **27**(1): 678-684.

Yannis S, Apostolos M, Loannis V, Angela-Gabriella D, Pasquale L, Emilio J, Pavlos CH, Valantis K, Athina B, Giovanni M and Spyridon K 2011. Antioxidant and anti-platelet properties of milk from goat, donkey and cow: An in vitro, ex vivo and in vivo study. *International Dairy Journal* **21**: 901-906.

Received 29 October, 2020; Accepted 08 February, 2021